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The Desiccation of Serum and other Protein Solutions, with Special Reference to the Reagents used in the Wassermann Reaction

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THE DESICCATION OF SERUM AND OTHER PROTEIN SOLUTIONS, WITH SPECIAL REFERENCE TO THE REAGENTS USED IN THE WASSERMANN REACTION.*

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Any method for the desiccation of serum, or other solutions containing substances of interest to the bacteriologist, must fulfil at least two conditions: the dried product must be soluble in water or normal saline, and the process must be one which inflicts little or no damage on the specific substances present in the original serum. The dried product, if required ultimately for injection into the human subject, should be sterile. If, however, the material is required for use in the laboratory for the study of serological reactions, then sterility, though desirable, is not absolutely essential.

One of the principal objects of the experiments described in this paper was the preparation of the serum reagents used in the Wassermann reaction in a stable form suitable for transport to isolated workers at home and abroad, where the difficulties of obtaining and preserving these substances are very great. It has been necessary to dry, at one operation, large quantities of serum; the method adopted has proved to be of great practical use and accordingly a description of the apparatus is given for the information of those workers who, though not perhaps specially interested in the reagents studied by us, may desire to desiccate large quantities of other fluids. This operation may be carried out in a variety of ways; large desiccators may be used, warm air may be fanned over thin layers of serum in large pans, or the proteins may be precipitated by means of ice-cold alcohol. (Hardy and Gardiner, 1910 [8]).

The method described below was evolved during an attempt made by one of us (P. H.) in 1909 to prepare large quantities of anti-rinderpest serum in the dry condition. The method described by Martin (1896 [10]) was tried but the results were not entirely satisfactory, failure being due to the choking of the filter candles used. This difficulty was overcome by substituting for the filter candle an ordinary separating funnel connected to a tube drawn out to a fine capillary. In other respects

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the method is similar to that of Martin, and is based upon the same principle: it is simple, convenient and inexpensive and involves only the use of apparatus available in any bacteriological laboratory.

Method.

Two large wide-necked bottles A and B (capacity 500 c.c. each) are joined in series by the tube C. The rubber stopper closing A is provided with three holes: through the centre one passes a piece of tubing which is drawn out to a fine capillary, the internal diameter of which is about 0·75 mm. This tube is connected to a separating funnel S by means of a short piece of pressure tubing. The separating funnel is held in position by means of a retort stand and clamp. G is a tin can fitted with a carbon filament lamp and is connected to the bottle A in the manner shown. This part of the apparatus is attached for the purpose

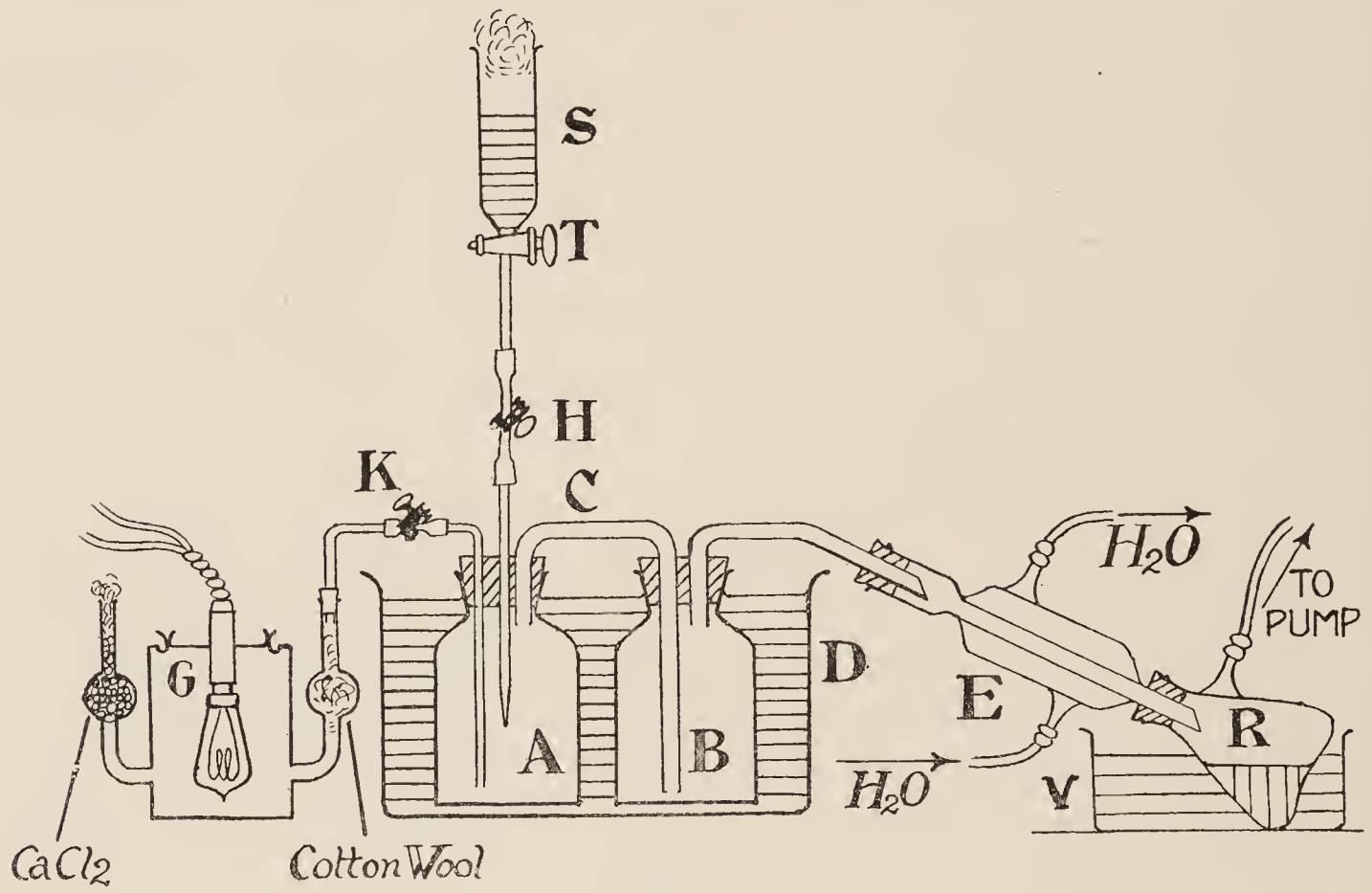


Fig. 1.

of supplying a current of warm, dry, sterile air, to assist, on occasion, in the drying process. Bottle B is attached to a double-surface condenser E, the delivery end of which is connected with a filter flask R which acts as a receiver. The bottles A and B are immersed in a water bath D, maintained at a constant temperature of 37° C., and the receiver is placed in a vessel V, filled with ice.

The receiver is attached to a water pump, and, all clips and taps being closed, the apparatus is evacuated. When the pressure has reached 20-25 mm. Hg (or lower) serum is admitted slowly by opening tap T and clip H. As the serum issues from the capillary tube it is converted into large bubbles or froth, which after a time fills bottle A and the excess passes over into bottle B. Water distils over and the solid constituents of the serum remain behind in the bottles in the form of yellow scales which are usually lightly adherent to the sides. When all the serum has been admitted it is advisable to allow the bottles to remain in the bath for an hour or longer, the vacuum being maintained meanwhile. Should the rate of inflow of serum exceed the rate of distillation, with consequent accumulation of liquid in bottle A, tap T is closed and a slow stream of warm, dry air is admitted by opening clip K until the excess is disposed of. In practice

it is seldom necessary to make use of this adjunct to the apparatus. The scales of dry serum are scraped off the sides of the bottle, transferred to a sterile Petri dish and stored in a desiccator containing sulphuric acid (or preferably phosphorus pentoxide) and the desiccator evacuated.

The apparatus will dry serum at the rate of about 100 c.c. per hour. For successful working it is essential that a pressure of 20-25 mm. (or lower if possible) be obtained and maintained. To secure this, a good pump must be used, and all rubber stoppers and connections must be tight. It has been found advantageous to use four bottles in series instead of two. It is not advisable to work at temperatures higher than 37° C., for although the rate of working is increased the solubility of the final product is adversely affected.

Somewhat similar methods have been described by Rosenau (1905 [21]) and by Burrows and Cohn (1918 [4]). Rosenau evaporated serum (diphtheria antitoxin) in a long tube warmed to 36° C. by bubbling warm dry air through it. There was some frothy bubbling at first, but this quickly subsided. Large sugar-like flakes of dried serum were obtained and there were no hard cakes. Burrows and Cohn evaporated serum (normal) in a large distilling flask containing glass beads, at a temperature of 50° C. They estimated the solid and liquid components of a number of sera. The dried products dissolved easily in water, the solutions being only slightly turbid.

Applications.

By the use of this method the following solutions have been reduced to the dry condition: anti-rinderpest serum, diphtheria antitoxin, tetanus antitoxin, anti-dysentery serum, solutions of albumin and globulin prepared from normal and immune serum, hæmolysin, normal human serum, and human serum reacting positively in the Wassermann reaction. The dried products thus obtained can be dissolved by the addition of the correct amount of water, and the solutions thus obtained apparently behave like the original fluid serum. The amount of immune body lost during the drying of sera is usually small and very often imperceptible.

The method has also been used for reducing to the dried condition Douglas's medium, the different fractions into which Witte's and other peptones can be separated, and litmus milk. The drying of this latter fluid proved to be the most difficult of any yet encountered, but the powder finally obtained, when dissolved carefully, served to differentiate the organisms of the enteric group just as well as the original fluid. In the case of such solutions as media, peptones, etc., a temperature higher than 37° C. may be used.

Solubility of Dried Serum.

One of the difficulties which has militated against the more common use of dried sera as reagents is the fact that frequently the products are not completely soluble in water. During our work on this subject we have reduced to the dry condition a very large number of

samples of serum and we have found that when dried *in vacuo* over sulphuric acid at the ordinary temperature the products are invariably easily and—to the naked eye—completely soluble. Serum which has been dried by the modified method of Martin also dissolves easily and yields a clear solution. At times, however, these latter solutions contain small insoluble white particles. The amount is never very large, and a few minutes in the centrifuge yields a supernatant fluid which can be used for any of the serological reactions which we have studied. We do not consider this slight insolubility—when it occurs—a serious defect, having regard to the many advantages which accrue from the use of such reagents.

An experiment was carried out to determine the solubility of dried serum and, at the same time, to investigate more thoroughly the nature of the solutions obtained when dried serum is redissolved in water. A perfectly clear amber-coloured serum, free from preservative, was used. A portion was dried in the desiccator and another portion by the frothing method. The theoretical amount of optically clear distilled water was added to each so as to reconstruct the original serum. Further, a portion of the dried serum obtained by the frothing method was ground to a fine powder in a mortar and a sample of this was also reconstructed. The solutions were examined and compared with the untreated control. Serum dried by the desiccator method dissolved easily; no particles could be seen and no sediment deposited even after long standing. The solution, however, was very slightly hazy. The degree of haziness was so slight that, had the original serum not been perfectly clear, it is probable that the difference would have been imperceptible to the naked eye. Serum dried by the frothing method also dissolved easily and apparently completely, but on standing for three days in the cold room a very slight sediment was observed which became more evident on gently shaking the tube. The amount of this insoluble material was determined in a special experiment and was found to be 0·09 per cent. The powdered serum was much less soluble, quite a heavy deposit settling down after a few hours. In this case the insoluble material amounted to 3·9 per cent. In three other experiments the same general results were obtained. It is evident that care must be exercised in the subsequent treatment of dried serum. The insolubility of fine particles of dried serum is referred to by Otto and Hetsch (1921 [16]).

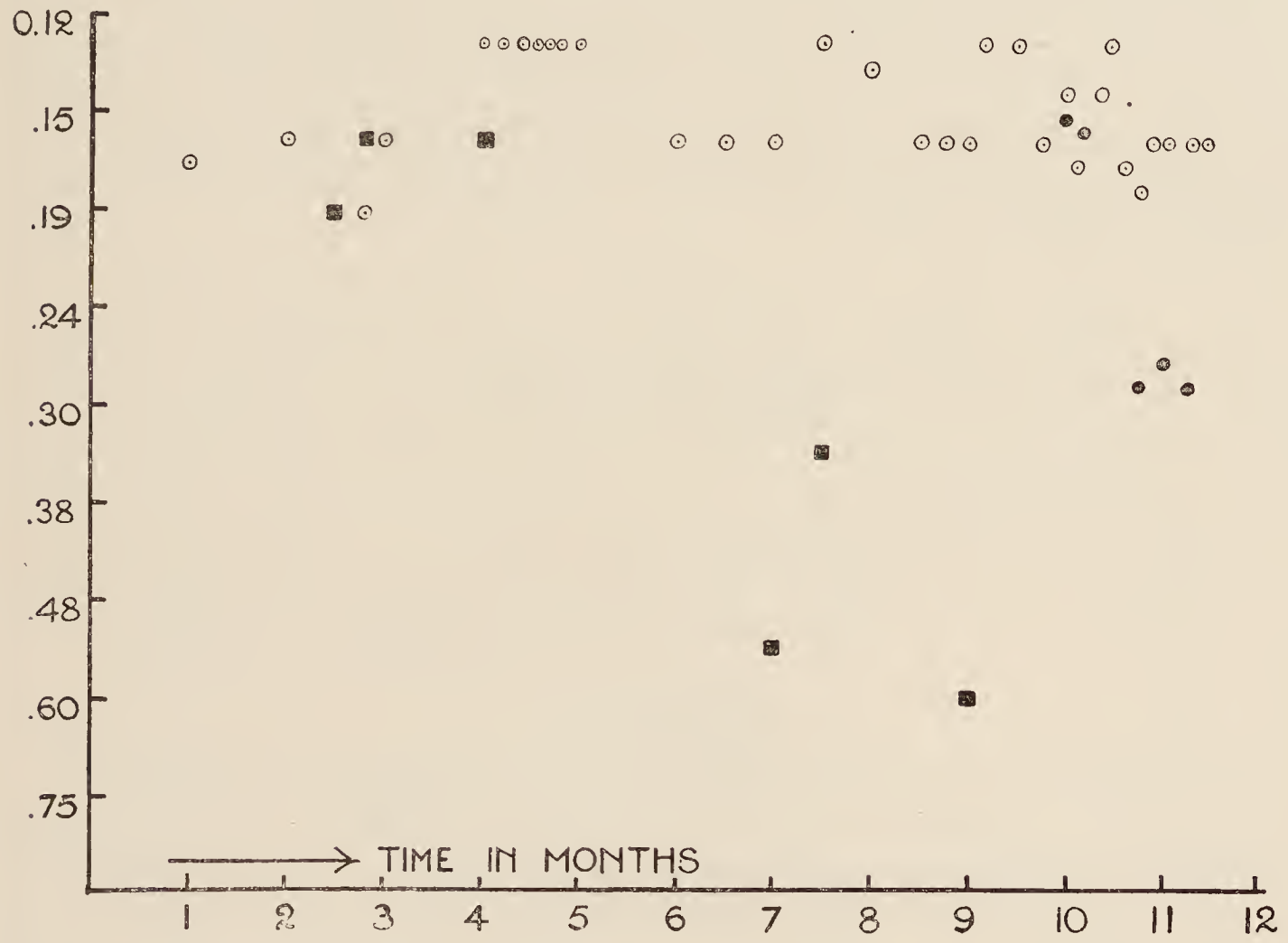
It was of interest to see to what extent the process of desiccation and re-solution had affected the physical properties and colloidal nature of the serum. In this study we have had the assistance of Mr J. E. Barnard of the National Institute for Medical research to whom we desire to express our thanks. The different solutions were examined by a method devised and used by Mr Barnard for comparing the relative size of particles in colloidal solution. It was found that the average size of the particles in the reconstructed sera was four times as large as those occurring in the untreated control, and it is interesting to note that, although there is such a marked change in the physical nature of the solutions, yet the immune sera which we have examined exhibit their specific properties which—as far as our methods of measurement can determine—are unchanged.

We have used the method and apparatus described above for the special purpose of reducing to the dry condition the reagents used in the Wassermann reaction, and have studied the properties and stability of the dried products so obtained.

Hæmolysin.

Hæmolysin, prepared by the injection of sheep's red corpuscles into horses, has been dried on many occasions with uniform success by the method described. The loss of immune body has rarely been detectable and the dried product when suitably redissolved behaves in the Wassermann reaction in precisely the same way as the original fluid serum.

Parallel tests have been carried out on many occasions with cells sensitised with liquid hæmolytic serum, and the equivalent amount of



Ordinates represent a tube scale; the first tube contains 1 c.c. of a solution prepared by dissolving 0·075 grm. dried hæmolysin in 100 c.c. saline, the amount in the subsequent tubes diminishing 20 per cent. from tube to tube. The figures represent the weight of hæmolysin (mgrms.) present in each tube.

⊙ = Hæmolysin kept at room temperature for 12 months; 32 observations made.

■ = Hæmolysin kept at 37° C. for 6 months; 6 observations made.

● = Hæmolysin kept at room temperature for 10 months, then at 37° C. for 1·5 months; 5 observations made.

CHART I.—Illustrating stability of dried hæmolysin at different temperatures.

dried serum redissolved in saline, and we have satisfied ourselves that both in complement and antigen titrations and in the Wassermann reaction the two reagents give identical results. In the titration of hæmolysin and complement, we used 1 c.c. of diluted hæmolysin, 1 c.c. of diluted guinea-pig serum, and 1 c.c. of a 2·5 per cent. suspension of washed sheep cells.

The stability of dried hæmolysin is illustrated in the above chart. Hæmolysin was dried in the manner described above and quantities of 0·075 gms. were weighed out. These were stored in the

laboratory (in a drawer) for twelve months, and at intervals a sample was tested, one of the weighed quantities of hæmolysin being dissolved in 100 c.c. of saline for each test. During the year thirty-two such tests were carried out, and it was found that during this time, and under these conditions of storage, the hæmolysin retained its activity and specific properties unchanged. Part of the material was placed in the incubator and kept at 37° C. Six determinations of hæmolytic activity carried out during a period of six months showed that under these conditions the hæmolysin gradually deteriorated in value. A further investigation of the stability of dried hæmolysin was carried out as follows: a number of the weighed samples after being stored at laboratory temperature for ten months, were placed in the incubator (37° C.). During the next six weeks five determinations were carried out and it was found, again, that at a temperature of 37° C. deterioration of the hæmolysin occurs.

TABLE I.

Effect of heat on ordinary fluid hæmolysin, and on the same hæmolysin when dried.

Hæmolytic Serum.	History.	Titre.
1.A . . .	Fluid, kept in cold room . .	1:1000
	,, ,, at 37° C., 28 days .	1:100
	Solid, kept at room temperature	1:1000
	,, ,, at 37° C., 28 days .	1:1000
2.E . . .	Fluid, kept in cold room . .	1:2440
	,, ,, at 37° C., 28 days .	1:100
	Solid, kept at room temperature	1:2440
	,, ,, at 37° C., 28 days .	1:1800
4.4 . . .	Fluid, kept in cold room . .	1:1950
	,, ,, at 37° C., 28 days .	1:60
	Solid, kept at room temperature	1:1950
	,, ,, at 37° C., 28 days .	1:1200

All the samples of hæmolysin that have been dried do not show this remarkable stability. Certain specimens have shown a more or less rapid deterioration, although in the process of desiccation there was no unusual loss of hæmolytic activity. In all the experiments we have carried out, deterioration has been much less marked than in the case of the corresponding fluid serum. Table I. shows the results of three such experiments. In each case fluid hæmolysin was preserved in the cold room for twenty-eight days and it was found that the original value of each serum was maintained during this time. A portion of each fluid hæmolysin was kept for the same period in the incubator at 37° C.: the loss in activity in the three cases amounted to 90, 96, and 97 per cent. respectively. The remainder of the serum was dried; part was kept at room temperature and the remainder in the incubator at 37° C. for twenty-eight days. The tests showed that dried hæmolysin when

stored at ordinary temperature maintained its value unchanged during this period, while of the three samples stored for twenty-eight days at 37° C. one showed no loss of activity, while the other two lost 25 and 40 per cent. respectively.

The complement titration is the most delicate test for the sensitiveness of a hæmolytic system. If the serum has not lost any potency during the process of desiccation, we have found that it acts precisely as the fluid of comparable strength. If it has lost power, there is a very slight reduction in the rate of the whole reaction, the same end point being reached but in a longer time. This phenomenon can only be elicited with difficulty; it is never very marked and does not detract from the practical value of the dried reagent.

Human serum, reacting positively in the Wassermann reaction, was reduced to the dry condition by this method, and it was found that the substance in such serum which confers upon it its specific properties can be dried without loss. A serum dried six years ago gives still a strongly positive Wassermann reaction. Normal human serum, reacting negatively in the Wassermann reaction has also been dried in the same way. Thus one can prepare in the dry condition and in a stable form positive and negative control sera for use in the Wassermann reaction. The control sera, when reconstructed, may be anticomplementary and should always be heated for ten minutes at 56° C. before use.

Complement.

Numerous attempts have been made in the past to preserve complement, and a variety of methods have been employed. Friedberger (1907 [5]) found that the addition of common salt preserved complement for several days, and Massol and Nowaczysnki (1910 [12]), Austin (1914 [1]), Thompson (1916 [25]), and Bigger and Wigham (1922 [2]) confirmed this observation. Different concentrations of salt were recommended by these investigators, who also showed that when such complement-salt mixtures were preserved in the dark and in the ice-chest the activity was preserved for several weeks. Rhamy (1917 [18]) showed that complement, when mixed with a 10 per cent. solution of sodium acetate, retained its activity practically unchanged for ten days. This result was confirmed by Ronchèse (1919 [20]) and by Hammerschmidt (1920 [7]). In a later communication (1918 [19]) Rhamy showed that if complement-acetate mixtures are preserved in the ice-chest the activity is retained for two to three months.

Browning and Mackie (1913 [3]), by freezing guinea-pig serum and maintaining it constantly at a temperature of −12° C. or less, found that its activity was retained to the full for a period of at least six weeks. Moledezky (1918 [13]) froze complement in an ice-salt mixture contained in a vacuum flask and stored the whole apparatus in the refrigerator. He states that under these conditions complement retains its activity

indefinitely. No details are given. Ruediger (1919 [22]) found that frozen complement retained its full activity for two weeks and then gradually lost strength. Later (1922 [23]), Ruediger found that frozen complement, stored at a temperature of zero Fahrenheit or lower, showed practically no deterioration in a month and that the complement remained active for eight to ten weeks.

Many observations have been made on the effect of desiccation on complement. Friedberger (*loc. cit.*) dried completely, *in vacuo* at the ordinary temperature, fresh guinea-pig serum and fresh serum to which common salt had been added. In each case he found that the dried complement was very stable. If the drying process was carried out at 37° C. there was a rapid loss in activity. He found that dried complement is extraordinarily resistant to heat. A temperature of 65° C. for one and a half hours, or 60° C. for fourteen hours, results in a loss of activity amounting to 50 per cent. Friedberger concluded from his experiments that while common salt preserves complement, in serum which is absolutely dry complement is thermostable. Noguchi (1907 [14]) found that complement did not disappear from serum dried at 23° C., the desiccated serum retained its complement activity for several months, and even after heating to 100°, 120°, and 135° C., dried sera, although weakened, did not lose completely their complementary action. When dissolved in the proper amount of water the solutions of dried complement were found to be thermolabile. Subsequently (1909 [15]) Noguchi described a method for carrying out the Wassermann reaction in which amboceptor, complement and antigen were dried on filter paper in a current of air at a low temperature. Shakell (1909 [24]) studied the desiccation of a number of tissues and sera—including guinea-pig serum—by a slightly different method. The serum was first frozen (by immersing the desiccator in a mixture of ice and salt) and then evacuated by means of a Geryk pump. The frozen state was maintained throughout the process. Shakell found that dried complement, prepared in this way, even after exposure to sunlight and the ordinary temperature for many weeks, retained its activity unchanged, as shown by its successful use in the Wassermann reaction. The products were perfectly soluble and yielded clear solutions. Massol and Grysez (1910 [11]) dried five sera in Petri dishes *in vacuo* and found that there was a rapid loss in activity during the process: the dried sera remained fairly stable for ten days, while after twenty days 80 to 90 per cent. of the original activity was lost. After the twentieth day little further decline in activity occurred. They consider that although dried complement may be useful when fresh guinea-pig serum cannot be obtained, yet it is always preferable to use the latter whenever possible. Massol and Nowaczysnki (1910) studied the keeping qualities of salt-complement mixture and dried complement and concluded that although there is much loss by the latter method the product obtained is very stable. Predtetschensky (1911 [17]) dried complement *in vacuo*

over anhydrous sodium sulphate and found that there was little loss in activity during the process, and that this dried complement could be used in the Wassermann reaction in just the same way as fresh guinea-pig serum. Predtetschensky states that his dried preparations can be used even after a period as long as two and a half years. Grigorowitsch (1913 [6]) added magnesium sulphate and common salt to guinea-pig serum and found that these salts exerted a preserving action on complement. He also dried serum treated in this way and found that the titre was unchanged after four months. Grigorowitsch states that he has used the combined methods of salt addition and desiccation for making a standard preparation. Karsner and Collins (1919 [9]) dried two specimens of guinea-pig serum by the method of Shakell; they found that their preparations failed to act in high doses (two and a half times the original dose) after eleven and fifteen days. They applied Shakell's method to the desiccation of other sera, but were unable to confirm his results.

Attempts were made by us to reduce complement to the dry condition by the method described above. The same result was obtained in three experiments, viz., there was a loss of activity which amounted to approximately 70 per cent. In each case the operation of drying occupied less than one hour and the temperature of the bath never exceeded 37° C. It is probable that the loss is due to mechanical action rather than to the heating at 37° C., for the frothing which occurs during drying resembles shaking, and it is well known that the activity of complement is markedly affected by the latter process. It is worthy of note, however, that although the loss of activity was so great that the reconstructed serum did not contain an adequate dose of complement for the Wassermann reaction when diluted 1/10, yet by using a larger quantity of the dried material the reaction could be carried out satisfactorily.

In carrying out any drying operation it is necessary to determine the total solids present in unit volume of serum. This may be done by pipetting out on to a weighed watch-glass exactly 2 c.c. of serum. The watch-glass and serum are then placed in a desiccator over fresh sulphuric acid and evacuated. The water is extracted from the serum very rapidly, but it is advisable to allow the desiccator to remain undisturbed until the following day. A second weighing gives the total solids in 2 c.c. of serum.

In the first experiment in which fresh guinea-pig serum was dried by the frothing method, total solids were determined on 2 c.c. of serum in the usual way. After the weight of the dried serum had been determined, 2 c.c. of distilled water were added; the dried serum dissolved easily and it was found that the titre of this reconstructed serum was exactly the same as that of the original guinea-pig serum which had been kept on the bench alongside the desiccator during the period required for the operation of drying, weighing and solution to be

completed (about eighteen hours). This result suggested that the simple drying of fresh guinea-pig serum *in vacuo* over sulphuric acid would prove to be a satisfactory and practical method. Accordingly, in the next two experiments larger quantities (30 c.c. and 60 c.c.) were treated in this way. Sterile Petri dishes (3 inches diameter) with level uniform bases were used, 10 c.c. of fresh guinea-pig serum being placed in each dish, and these were accommodated in a large desiccator (diameter 9 inches) containing fresh sulphuric acid and evacuated. Next morning the dried scales of serum were removed and it was found again that the operation had been quite successful in so far as the product was quite soluble and there was no loss of activity in one case, and very little in the other. Since the end of last year ten specimens of complement, averaging about 30 to 40 c.c. of pooled guinea-pig serum, have been dried successfully in this way. Some of this material was given to us by the Lister Institute of Preventive Medicine, to whom we wish to express our thanks.

TABLE II.

Date.	Volume of Guinea-pig Serum Used.	Method of Drying.	Weight of Total Solids in 1 c.c. Serum.	Loss in Activity Per Cent.
25.10.21 . .	40 c.c. 2 ,,	Frothing Desiccator	70 mgrms.	70 Nil.
2.11.21 . .	43 c.c. 30 ,,	Frothing Desiccator	65 mgrms.	70 Nil.
18.11.21 . .	70 c.c. 60 ,,	Frothing Desiccator	70 mgrms.	70 ? 6

The essential condition for success is rapid drying of the guinea-pig serum and this is secured by using a good pump, a desiccator which maintains the low pressure primarily produced and the use of fresh sulphuric acid. It is possible that even better results would be obtained by the use of phosphorus pentoxide and experiments on this point are in progress. It is not advisable to stack the Petri dishes in the desiccator.

Experiments were next carried out to investigate the stability of dried complement. About 4 grms. of material were obtained in one experiment (18.11.21) from serum which contained 70 mgrms. of solids per cubic centimetre. Small tubes, 10 c.m. long and 8 to 10 mm. internal diameter were used, 70 mgrms. of dried complement being placed in each. The tubes were then sealed in the blowpipe and stored in the laboratory at ordinary temperature. The titre of the original serum, and of the same immediately after drying was determined and during the next nine months the titre was determined on thirteen occasions, a tube being opened when required and exactly 1 c.c. of distilled water added. The water was allowed to stand with the serum

for ten to fifteen minutes and then the whole was stirred gently with a very thin glass rod until solution was complete. The results of this experiment are shown in the following chart.

During the first two months there is little change in activity. Deterioration then sets in and at the end of four months the value is reduced to about one half. It is interesting to note, however, that with this particular sample of dried complement there was no further decline in value during the next five months. This result has been obtained on other occasions. Thus, another sample of dried complement,

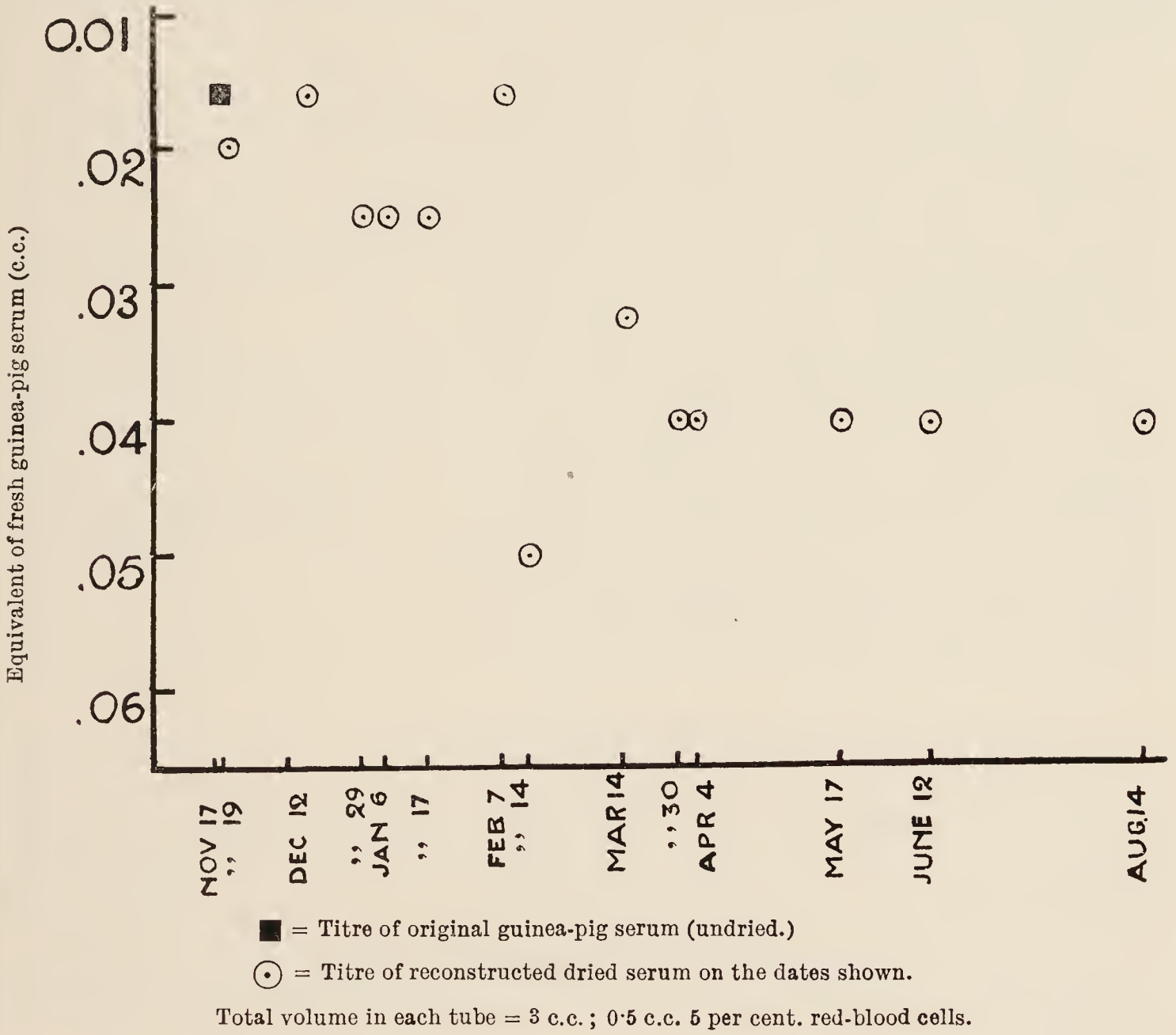


CHART II.—Illustrating stability of dried complement.

the original titre of which was 0·015 c.c., had a value of 0·032 c.c. after being kept—partly at room temperature and partly in the cold room—for $9\frac{1}{2}$ months. It is also of interest to record that another sample of dried complement, sent to Uganda, was successfully used there for the purpose of the Wassermann reaction. It is possible that the deterioration which occurs is due to traces of moisture which the dried serum contains, and that if the moisture content be further reduced the dried complement may be found to be even more stable. Further, although in six months the activity falls to one-half, yet even then the dried complement when suitably reconstructed is still serviceable. The dried

complement, when reconstructed with water, behaves in all respects like fresh guinea-pig serum and is equally susceptible to heat. Complement six months old, in appropriate dosage, has been used satisfactorily in the Wassermann reaction and other hæmolytic tests.

It is believed that these dried reagents for hæmolytic tests may prove of considerable use in routine work. Dried horse hæmolysin appears to be in every way as satisfactory as fluid hæmolysin for complement fixation tests, and it has the additional advantage of much greater stability. By using dried positive and negative Wassermann sera, control sera of exactly the same activity can be included in the tests over a prolonged period of time.

Dried guinea-pig serum provides a means by which active complement will stand postal transit and can be made available to workers who are unable to obtain fresh guinea-pig serum, and although we do not claim that dried complement possesses all the advantages of fresh guinea-pig serum, it can at least be made to give perfectly dependable Wassermann results when properly used.

In particular, we hope that these dried reagents may prove of use to isolated workers in the tropics, and other places where the difficulties of obtaining and keeping Wassermann reagents are great. Quite apart from this, the possibility of obtaining in a relatively stable form so many of the Wassermann reagents, including complement, should prove of considerable service in the standardisation of the Wassermann reaction and in other hæmolytic research.

Summary and Conclusions.

(1) Serum, and other reagents of bacteriological and immunological importance, can be dried with little or no loss of their specific properties.

(2) With the exception of complement, most of the reagents investigated can be conveniently dried by a modification of Martin's method.

(3) Guinea-pig serum can be dried successfully in a good desiccator, and the complement activity of the dried product is relatively stable.

(4) Horse hæmolysin and positive Wassermann sera are very stable when dried.

(5) Dried hæmolysin, positive and negative Wassermann sera and complement may prove of value to isolated workers and to those engaged in research work, particularly on the subject of standardisation of the Wassermann reaction.

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